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ABSTRACT The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a major coleopteran pest in flour mills and storage facilities. An aggregation pheromone has been identified for this pest; however, the pheromone is of limited value for population monitoring. To develop more efficient methods to monitor this pest, experiments were conducted to determine whether light functioned as an attractant for the red flour beetle. Light-emitting diodes (LEDs) of various wavelengths were examined as light sources because they produce bright, narrow light spectra. A comparison of responses to light spectra across the visible and UV regions of the electromagnetic spectrum indicated that the beetle was most attracted to near UV LED at a 390 nm dominant wavelength. The use of LEDs in competitive laboratory experiments resulted in a 20% capture of released beetles, compared with a 1% capture with the aggregation pheromone alone. Even more beetles were captured with a combination of LEDs and commercially available chemical lures in traps. LEDs can easily be added onto existing trap designs or new traps can be designed to take full advantage of positive phototaxis.

KEY WORDS pitfall trapping, stored product, LED, insect vision

Tribolium flour beetles, particularly the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and confused fluor beetle, *Tribolium confusum* Jacquelin du Val, are important pests of stored products. The male-emitted aggregation pheromone of *T. castaneum,* was first suggested by Ryan and O'Ceallachain (1976) and then isolated and identified by Suzuki and Sugawara (1979). This aggregation pheromone, 4,8-dimethyldecanal, has since been shown to be a relatively weak attractant in mill trapping situations (Chambers 1990). Recently other chemicals released by beetles have also been shown to be attractive to *T. confusum* but the active space of the compounds has not been determined and their activity in *T. castaneum* is repellent instead of attractive (Verheggen et al. 2007; A.J.D., unpublished. data). The typical flour mill habitat of the red flour beetle is a complex environment filled with food odors and is therefore a difficult environment in which to deploy a pheromone-based lure. A long-distance attractant would aid in bringing individuals close enough to be attracted by the aggregation pheromone and would greatly increase trap catch. Development of more effective trapping systems for flour mill environments will provide better insect surveillance and enable more accurate temporal and spatial treatment options to control pest populations.

Previous research examined the attraction of stored-product pests to various wavelengths of light. Only certain wavelengths can be perceived by red flour beetle because it lacks the blue (B)-opsin that detects wavelengths between 400 and 500 nm (Jackowska et al. 2007). When a selection of wavelengths was presented, green light was most attractive to red flour beetle, although the dominant wavelengths did not include those shorter than 400 nm (Stermer 1959). In shed trapping experiments, a mixture of green and UV light was most attractive to red flour beetle (Kirkpatrick et al. 1970, Soderstrom 1970); however, fluorescent lamps emitting relatively broad ranges of wavelengths were used in these experiments. In a more recent comparison of incandescent light sources, a preference for white light over blue and green, with a repellence from red was reported previously (Sheribha et al. 2010).

Insects have highly conserved visual pigments with maximal spectral absorption between 350 and 550 nm (Briscoe and Chittka 2001), this allows for vision in the UV range of the electromagnetic spectrum. red flour beetle as a species lack the ability to see the middle portion of this common visual range (Jackowska et al. 2007). Based on visual cues and behavioral tendencies, bees are more attracted to flowers reflecting near UV light, whereas beetles are more likely to pollinate red flowers (Briscoe and Chittka 2001). The evolutionary history and physiological needs of individual species determines the wavelengths that are attractive or repellant (Briscoe and Chittka 2001). Flour is an ideal habitat for red flour beetle and, as such, its spectral

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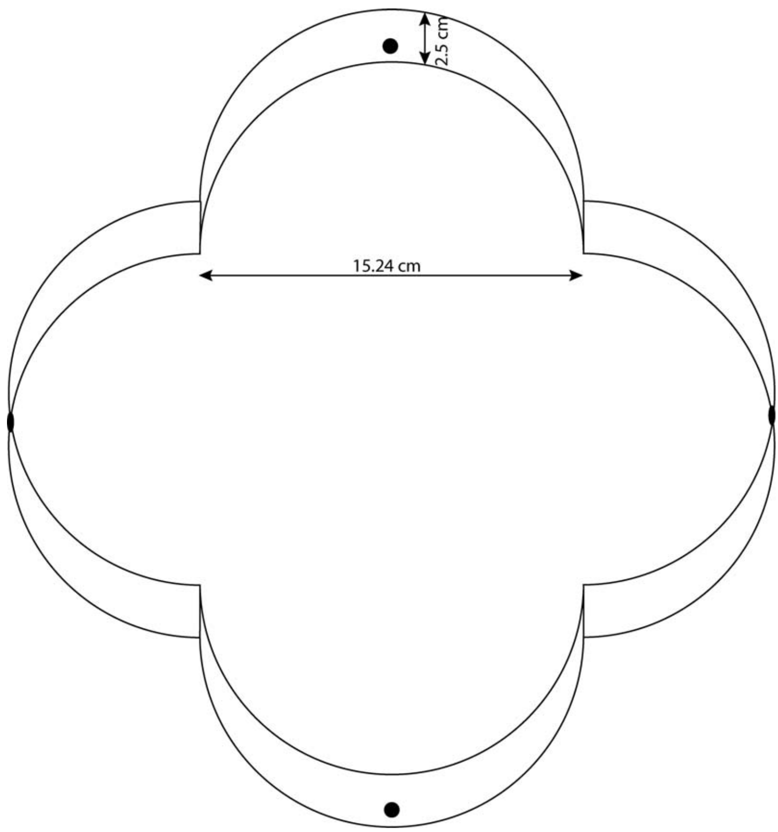

Fig. 1. Diagram of the cloverleaf arena, it was made from aluminum flashing material with LED holders at the apex of each leaf, represented by black circles. LEDs were adjusted to emit 10 mW/cm^2 by using potentiometers.

reflectance peak at wavelengths slightly shorter than 400 nm (Zandomeneghi et al. 2000) may be particularly attractive for red flour beetle.

Narrow wavelengths of light, tuned to the phototaxis of specific insect species have been known to increase trap capture of mosquitoes (Burkett et al. 1998) and phlebotomine sand flies (Cohnstaedt et al. 2008). Currently light traps are used to catch Diptera and nocturnally active insects in flour mills. These traps use broad spectrum fluorescent bulbs and consequently are bulky and must be attached to mainline power. Light-emitting diodes (LEDs) use less energy and may be selected to produce narrow spectral bands of light. The solid state physics of LEDs allows light traps to be deployed in harsh and varied environments for population monitoring and control. Additionally, LEDs are often smaller, lighter (in weight), and longer lasting than traditional fluorescent or incandescent lamps. Adding color-specific LEDs to traps designed for crawling insects might enhance surveillance, increase trap life, and lower operating costs all at the same time.

This series of experiments was conducted to determine the visual preferences of the red flour beetle. They were evaluated in three ways: 1) wavelength preference, i.e., are specific wavelengths attractive; 2) deployment preference, i.e., does the location of LEDs on a trap influence trap capture; and 3) pheromone synergy testing, i.e., does a chemical attractant increase trap capture?

Materials and Methods

Three experiments were conducted to determine whether red flour beetle were attracted to specific wavelengths of light. The first experiment tested the attraction of individuals to single-color LEDs. The second experiment tested trap designs to determine optimal LED deployment, and the third experiment examined the increase in trap efficiency due to the addition of LEDs to traps and the effect of chemical attractants.

Arena Spectral Preference Assays. LED attraction testing occurred in an arena made of flashing material cut to a 2.54-cm strip and then bent into four connected half circles, each with a diameter of 15.24 cm (Fig. 1). The two ends were connected with a #10 stainless steel machine screw and a locking nut. At the furthest point from the center of each half circle a hole was drilled and a 5-mm LED holder (part no. 276-080, Radio Shack Corp., Fort Worth, TX) was inserted with its midpoint 0.79 cm from the bottom of the arena. This design put the center of the LED closer to the floor of the arena. The inside of the arena was coated with

matte black spray paint to reduce reflection across the arena so as to prevent confusing the beetles. The arena will be referred to as the cloverleaf arena and its shape was developed because beetles following the edge reenter the center of the arena as they move from one leaf across the 90° angle between leaves.

An LED infrared light source (Tracksys LTD, Nottingham, United Kingdom) and an infrared video camera (WV-BP330 Panasonic CCTV camera, Panasonic Corp., Secaucus, NJ) were set up above the arena to record beetle movement in the dark using MEDIACRUISE software (Canopus Corp., San Jose, CA). This enables red flour beetle behavior in the area to remain visible to observers. Light emitting diode intensity was calibrated so each emitted 10 mW/cm^2. These LEDs ranged from red to UV, which spanned the visible spectrum. First, 410 nm (blue), near UV (390 nm), and UV (380 nm and 360 nm), were tested competitively, and the most attractive was compared with LEDs from the remaining visible range (655 nm [red], 555 nm [green], and 587 nm [yellow]). Preliminary tests of beetle movement toward near UV LEDs, white LEDs, or unlit LEDs, indicating the possible attraction of the near UV and are the reason for the development of the competitive LED preference assays.

The red flour beetle tested in this experiment were taken directly from laboratory cultures at CMAVE at 1–2 wk posteclosion. In each trial, 10 *Tribolium* were released into the center of the arena, and their movements were recorded for the 5 min immediately after their release. The number of beetle visits to LED lights from the center of the arena was visually determined. This ensured all recorded attractions were individuals moving from the center of the arena toward an LED as opposed to following an edge past an LED. Each individual was able to visit an LED more than once, and so each visit to a LED in the 5-min test was scored. As is the habit of *T. castaneum*, all individuals moved around the arena in each trial. Ten replicates were carried out for each of the two groups of LEDs tested. Analyses of variance (ANOVAs) were used to measure the statistical differences in LED visits, specifically proc GLM by using the Tukey test to compare the individual means (SAS Institute 2004).

Trap LED Deployment. The second experiment was carried out between May and July in Gainesville, FL, in a shed (8.99 m in length, 3.05 m in width, and 2.29 m in height). All visible cracks were sealed with caulk, and the linoleum tile floor was sealed with concrete sealer. A second panel was attached to the inside of the door to completely block any light that might come in around the edge of the door. The average temperature in the shed was 29.4 ± 1.75°C, and the average relative humidity was 70.7 ± 5.51%. The temperature was controlled with a thermostat and air-conditioning unit. For each trial, 300 unsexed beetles between 1 and 2 mo old were released into the shed. Between each of the five trials the shed was vacuumed to remove remaining individuals. Each trial lasted 24 h. For each trial the release points of the insects, the location of each of the three traps, the number of individuals caught in each trap, and the number of individuals around but not within each trap were recorded.

The traps were Trécé Dome traps with Trécé RFB/CFB Storgard caps (Trécé, Adair, OK). Traps were LED-modified in either of two fashions: one UV LED disc (eight LEDs) from the BioQuip CDC trap (catalog no. 2770, BioQuip, Rancho Dominguez, CA) was mounted onto the top of the trap or six separate 5-mm LEDs (Fox Electric Supply, Philadelphia, PA) were added around the bottom of the Dome trap. To add LEDs to the bottom of the trap, holes were drilled through the bottom of the Dome trap, and LEDs were hot glued in place. The LEDs used were rated at 390 nm with a band width of 385–395 nm with an optical power rating of 10 mcd per LED. A 6-V battery was used to power the LEDs in both cases, for the top mount array the battery was placed on a small wooden stand 5 cm above the top of the Dome trap and for the bottom mounted individual LEDs the battery sat directly on top of the trap. These two trap designs, and an unmodified control Dome trap, were tested competitively in the second experiment, and all traps were placed in the shed for each trial. The traps were placed along the center line of the long axis of the room, evenly spaced from each other (2.25 m apart) and the end walls, trap locations were rotated between each of six trials. ANOVA with an analysis of covariance (ANCOVA) was used to determine the best trap configuration with trap location as a covariate, proc GLM was used with the cov keyword, and the Tukey test to compare the means of the individual treatments (SAS Institute 2004).

Trap Test With Chemical Attractants. In the third trial, the more successful of the two LED light trap designs, the top mount, was deployed along with an unmodified Dome trap to directly measure the increased catch due to the addition of the LED array. This assay was conducted five times with Trécé RFB/CFB Storgard caps in both traps and five times without the caps. Like the other trials, the traps were placed along the long axis of the room evenly spaced from each other (3 m apart) and from the walls, trap location was rotated between trials, and 300 beetles were released per trial. Data collected included both beetles captured in the center pitfall area of the trap and also beetles within 0.5 m of the trap when the 24-h sampling period ended. *t*-tests were used to demonstrate the effectiveness of the lighted traps against controls within the baited and unbaited trials (Cochran method because of unequal variances) and to examine the effect of the chemical attractant on the number of insects inside and around the traps between trials (pooled method because variances were equal; SAS Institute 2004).

Results

Arena Spectral Preference Assays. The first laboratory experiment indicated red flour beetle movement was significantly affected by LEDs ($F_{3,36} = 34.00$; $P <$

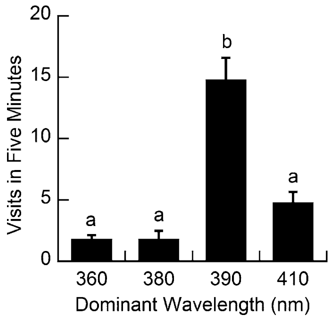

Fig. 2. Response, measured by an individual walking toward an LED, to blue and near UV spectra by 10 red flour beetle over 5 min in a cloverleaf shaped arena (n = 10). Error bars denote SE, and significant differences (α = 0.05) by least squares means are indicated by letters ($P <$ 0.0001).

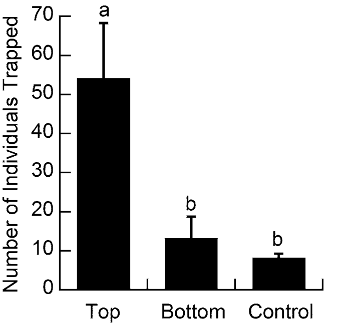

Fig. 4. Number of red flour beetle caught in Dome traps either with LEDs at the top, bottom or without LEDs. Three hundred individuals were released and then trapping occurred over a 24-h period (n = 6). The trap with LEDs at the top was the most effective at trapping red flour beetle. Error bars denote SE and significant differences are indicated by letters ($P <$ 0.001).

0.0001) and of those emitting in the blue to UV range tested the 390 nm was the most attractive (in the cloverleaf arena) at $P <$ 0.0001 (Fig. 2). The second set of trials examined how the most attractive wavelength in the blue to UV range compared with LEDs emitting light in other parts of the visible spectrum. The statistical model was again highly significant ($F_{3,36}$ = 26.52; $P <$ 0.0001) and the response to the near UV 390 nm LED indicated it was also more attractive than segments of the visible spectrum at $P <$ 0.0001 (Fig. 3).

Trap LED Deployment. The results of the cloverleaf trials indicated that out of the LEDs tested the one that emitted light at 390 nm was the most attractive to red flour beetle in the laboratory (Figs. 2 and 3). Trécé Dome traps were then augmented with 390 nm LEDs in a shed. LED location on the trap was highly significant ($F_{2,15}$ = 7.77; P = 0.0048). A trap with LEDs at the top was more attractive than either a standard trap (P = 0.011) or a trap with LEDs at the base (P = 0.0093; Fig. 4). Although red flour beetle move toward near-UV light, these results indicate that they move toward but not directly to those LEDs.

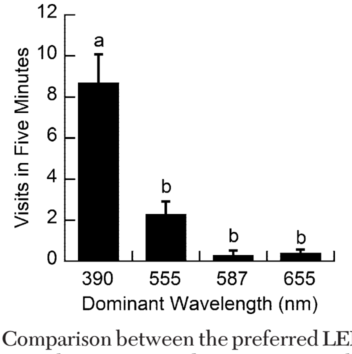

Fig. 3. Comparison between the preferred LED from the near UV range, the 390 nm, and LEDs emitting light in the visual portion of the electromagnetic spectrum. The number of times individual red flour beetle walked toward an LED emitting each wavelength over 5 min was recorded in the cloverleaf arena (n = 10). Error bars denote SE, and significant differences by least squares means are indicated by letters ($P <$ 0.0001).

Trap Test With Chemical Attractants. The most attractive trap setup, the top lighted, was then tested competitively with the standard Dome trap, this was replicated with and without the addition of Trécé red flour beetle pheromone septa to both treatment and control traps. The top light trap continued to be very attractive and much more attractive than the standard Dome trap, both in baited and unbaited trials when insects both in and around the traps were counted together (t_9 = −10.81, $P <$ 0.0001), similarly the lit trap captured more beetles when the individual baited and unbaited trials were compared with their controls (baited: t_4 = −11.07, $P <$ 0.001; unbaited: t_4 = −11.08, $P <$ 0.001). When only the lit traps were considered in both baited and un-baited trials, baits affected the number of beetles entering the pitfall area of the trap (t_8 = −3.70, P = 0.006) but not the number of beetles near the trap (t_8 = 1.06, P = 0.3201). All trials were conducted in the dark where the only source of light was an LED array mounted on the Dome trap.

Discussion

Certain light wavelengths were more attractive to red flour beetle than others, and recent increases in LED technology both in reduced power consumption and in wavelength specificity make it possible to use light in small inexpensive traps deployed independently from mainline power. Although a specific light wavelength was effective at drawing red flour beetle close to traps, a chemical attractant increased trap entry. The number of insects near but not inside the trap indicated that the pheromone and visual attraction were not optimally bringing insects into the trap. Additionally the bait was not effective in altering the number of individuals near the trap although it did bring more into the trap. The Dome trap was used in these experiments, but alternate trap designs need to

be investigated to more efficiently guide target insects into the pitfall area by optimizing visual attraction and thigmotactic cues. Using a black trap with a columnar profile against a lighter background will likely increase visual attraction and the impact of the LEDs (Semeao et al. 2011).

Most research into red flour beetle attraction has focused on aggregation pheromones such as 4,8-dimethyl decanal. The nonselective mating habits of red flour beetle, that is their willingness to attempt copulation with beetle shaped lumps of flour, indicates visual attraction is not very important in finding mates and conspecifics (Fedina and Lewis 2008). Red flour beetles are long-lived insects that occur in ephemeral habitats; therefore, red flour beetle must find new food sources over the course of a lifetime. Beetles have to disperse to find new habitat (Sokoloff 1972), and spectral reflectance may provide an important visual cue. *Tribolium* species have highly developed eyes, very similar to the eyes of some Diptera and so have good visual acuity (Sokoloff 1972). Dispersing to new habitat requires recognizing food resources from long distances relative to their size. Visual perception of those food resources could rapidly allow red flour beetle to find a preferred habitat and determine optimal patches. Although red flour beetle are able to survive on many different substances, cracked cereal grains are a preferred resource. Flour has a high reflectance at 390 nm, whereas shorter wavelengths are absorbed by luteins and longer wavelengths are absorbed by pigments such as carotenoids (Zandomeneghi et al. 2000). Thus, 390 nm light attraction would be advantageous for foraging. Other experiments with the small hive beetle, *Aethina tumida* Murray, show that attraction to this wavelength may be related to dispersal and finding open spaces (A.J.D., unpublished data), so beetles coming from culture jars would be particularly likely to use this cue for dispersal.

Other insect species also are attracted to near UV and UV wavelengths (Stermer 1959, Kirkpatrick et al. 1970, Cohnstaedt et al. 2008); however, the degree of attraction shown by *T. castaneum,* an insect not strongly attracted to chemical cues, to traps with this wavelength of light, is significant for the stored product industry. Furthermore, we found that other beetles are attracted to UV wavelengths. When light traps baited with *Tribolium* pheromones were placed in a shed for preliminary testing, they also caught drugstore beetles, *Stegobium paniceum* (L.), from a previous experiment (A.J.D., unpublished data). This suggests that the attractiveness of this type of trap is not limited to red flour beetle and may be effective capturing other stored product insects. The ability of LEDs to emit a very narrow range of wavelengths enabled these trials to more closely address attraction to specific wavelengths. Past research by chance missed the dominant wavelengths most attractive to red flour beetle (Stermer 1959), whereas other studies used bulbs that emitted a spectrum of wavelengths too broad to focus in on the specific wavelengths attractive to red flour beetle (Soderstrom 1970, Sheribha et al. 2010). Our laboratory and shed trials indicate that light trapping using new LED technology will improve the efficiency of stored product pest capture and increase surveillance efficacy. This study focused on demonstrating trap effectiveness relative to established traps in controlled, dark environments. The visual complexity of a flour mill will make it more difficult to ensure sufficient contrast between the trap and the background. Trap placement, in dimmer or shadowed areas and along dispersal corridors, will probably increase trap effectiveness.

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